REMARKS

The Claimed Invention

The claimed invention is directed to xanthene derivative dye compounds that include additional fused rings, methods of using the claimed compounds and kits comprising the xanthene derivative dye compounds. The compounds may be present in a form containing a chemically reactive group, or attached to a biological compound or present as a non-reactive dye compound. These dye compounds, in their various forms, are used to stain or label a biological sample wherein the fluorescent compounds provide a detectable signal that identifies aspects of the biological sample.

The Pending Claims

Prior to consideration of the following Response to Office Action, Claims 1-22, 24-41, 43-44 and 46-54 are pending; Claims 23, 42 and 45 having been previously cancelled. Claims 1-21 have been withdrawn from consideration. Claims 22-39 are directed to dihydrohydroxyquinoline compounds of the invention. Claims 40-41, 43-44 and 46-48 are directed to methods for staining a biological sample using compounds according to Claims 22 and 24-39. Claims 49-54 are directed to kits comprising compounds according to Claims 22 and 24-39.

The Office Action

Claims 1-22, 24-41, 43-44 and 46-54 are pending.

Claims 1-21 have been withdrawn from consideration.

Claims 40-44 and 46-54 have been allowed.

Claim 22 stands rejected under 35 USC 112 first paragraph for failing to provide written description and second paragraph for containing indefinite claim language.

Claims 22 and 24 stand rejected under 35 U.S.C. 102(b) as being unpatentable over Lee et al. (6,080,852).

Claims 22, 24, 25 and 28-33 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al. (6,080,852).

Claims 26, 27 and 34-39 stand objected to as being dependent upon a rejected base claim.

Amendments

Claim 22 has been amended to replace the claim language "comprising a" with "of the" as recommended by the Examiner.

Claims 22, 40 and 49 have been amended to correct a typographical error in the structure as drawn. The added structure accurately represents the correct ring bonds. Support can be found on page 21, lines 10-14; page 22, lines 15-20 and page 23 lines 13-15.

Claims 22, 40 and 49 have been amended to clarify that E, E', X or X' do not represent $CR^1=CR^2$.

Claim 34 has been amended to correct a clerical error.

Claim 35 has been amended to correct a typographical error.

Applicants believe that no new matter has been added by any of these amendments and the Examiner is respectfully requested to enter them.

RESPONSE TO THE RESTRICTION REQUIREMENT

In the response that follows, the Examiner's individual comments are provided in full text, as identified by indented small bold print, followed by the Applicant's comments.

Applicant's election with traverse of Group V in Paper No. 7 is acknowledged. The traversal is on the ground(s) that Groups I to V should be rejoined because Group I represents compounds which are intermediate compound used to synthesize the compounds of Groups II, III, IV and V. This is not found persuasive because the compounds of Group I have a separate utility other than to make the compounds of Groups II, III, IV and V. The compounds of Group I can be used to make compounds which are structurally different from the compounds of Groups II, III, IV and V.

The requirement is still deemed proper and is therefore made FINAL.

Claims 1 to 21 stand withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention. Applicant timely traversed the restriction (election) requirement in Paper No.7.

The method claims of Group VI (claims 40 to 44 and 46 to 48) will be regrouped with the elected compound claims of Group V (claims 22 and 24 to 39). New claims 49 to 54 drawn to a kit for staining a sample will also be examined with the elected claims.

Applicants acknowledge that Claims 1-21 have been withdrawn from consideration and greatly appreciate that the Examiner has regrouped Groups V and VI as well as including the newly added kit claims, all of which are to be examined with the elected claims.

RESPONSE TO THE REJECTIONS

In the response that follows, the Examiner's individual rejections are provided in full text, as identified by indented small bold print, followed by the Applicant's response.

35 U.S.C. 112, 1st ¶ Rejection

Claim 22 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

There is no antecedent basis in the specification for the new formula, which was inserted in claim 22. The bonding in the formula is different from the bonding in the formula shown on I page 21, lines 10 to 14.

Claim 22 has been amended to correct the typographical error in the structure as drawn wherein the correct ring bonding is now depicted. Applicants respectfully assert that the newly drawn structure is supported by the specification and represents the Applicant's present invention.

Applicants believe this claim amendment overcomes this rejection and the Examiner is respectfully requested to withdraw the rejection based on 35 U.S.C. 112, first paragraph.

35 U.S.C. 112, 2nd ¶ Rejection

Claims 22 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The use of the term "comprising a" in a compound claim is indefinite since the structural formula represents complete compounds and not radicals it is not known how the compound can comprise something else since there is no free valence to bond anything else. To overcome this rejection the term "comprising a" should be changed to -of the -.

As recommended by the Examiner, Claim 22 has been amended to remove the language "comprising a" with the proposed definite claim language "of the". Applicants believe this claim amendment over comes this rejection and the Examiner is respectfully requested to withdraw the rejection based on 35 U.S.C. 112, second paragraph.

35 U.S.C. 102(b) Rejection

Claims 22 and 24 are rejected under 35 U.S.C. 102(b) as being anticipated by Lee et al. (US 6080852), cited.

The reference discloses the claimed compound wherein Q is CR^{28}, R^{28} is the phenyl radical wherein R^{30} is carboxylic acid group, R^{34} and R^{31} are Cl and one of R^{32} and R^{33} is carboxylic acid and the other is hydrogen, R^1, R^2, R^3, R^4, R^5, R^{41}, R^{42}, R^{43}, R^{44} and R^{46} are hydrogen, R^3 and R^{45} are methyl, one of E and X is $CR^1=CR^2$ and one of E' and X' is $CR^1=CR^2$ where R^1 and R^2 are hydrogen. Note Example 4.

Applicants have amended Claim 22 wherein E, E', X or X' no longer represent "$CR^{1'}=CR^{2'}$". The substitutents, E, E', X or X' are only permitted to represent the heteroatoms, O, S or N wherein the claim limitations result in a five-membered ring. The '852 patent only discloses that the corresponding ring structure comprise carbon ring atoms, not heteroatoms such as O, S or N, or that the corresponding ring be 5-membered instead of 6-membered. Therefore, due the structural differences of the presently claimed compounds, Applicants respectfully assert that the presently claimed compounds do not fall within the scope of the '830 patent.

Applicants believe the Claim amendment to Claim 22 over comes the anticipation rejection of Claim 22 and 24 over the '852 patent and the Examiner is respectfully requested to withdraw the rejection.

35 U.S.C. 103(a) Rejection

Claims 22, 24, 25 and 28 to 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al. (US 6080852).

This rejection is respectfully traversed because the cited reference (the '852 patent) does not teach all the presently claimed limitations.

Determination of the scope and content of the prior art (MPEP §2141.01)

The reference discloses structurally similar compounds which are used to dye nucleotides and in polynucleotide sequencing. Note Example 4.

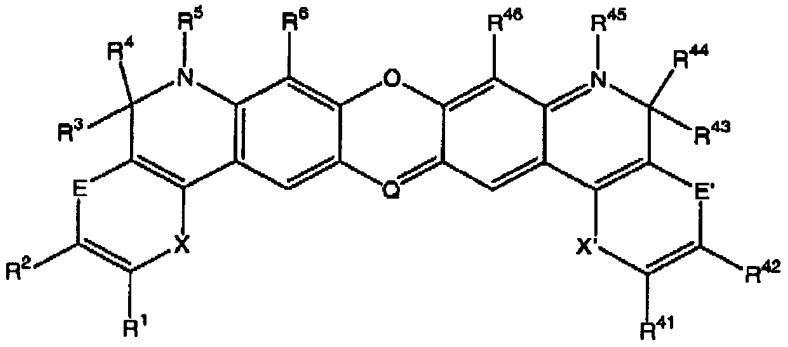
The Applicants respectfully assert that the presently claimed invention no longer encompasses the compound in Example 4, or any of the compounds disclosed in the '852 patent. Thus, the presently claimed compounds are not structurally similar to the compounds disclosed in the '852 patent.

Ascertainment of the difference between the prior art and the claims (MPEP §2141.02)

The claimed compounds which are position isomers, homologs or close structural analogs of the compounds disclosed by the reference would have been rendered obvious. For example, the claimed compound which is like the compound of Example 4 of the reference except that the methyl groups are replaced by ethyl groups is a homolog.

The compounds of the reference also differ from the compounds of claims 25 and 28 to 33 in that they are not specifically substituted with a group L-Sc where Sc is a protein, polypeptide, nucleoside, nucleotide or polymer. However, the reference discloses that the dyes can be linked to a proteins, polypeptides, nucleosides, nucleotides and polymers. Note column 11, line 40 to column 16, line 2.

Applicants respectfully assert that the presently claimed compounds are not position isomers, homologs or close structural analogs as one of skill in the art would understand these terms. This is primary due to the fact that the presently claimed compounds do not permit the lower fused ring structure to be a 6-membered carbon ring; the claims require that the ring structure comprise at least one O, S or N atom in a 5-membered ring. The compound having the following structure:



wherein E, X, E' or X' is O, S, NR^8, provided that E and X or E' and X' are not both present.

Fluorescent dyes are generally known in the art as being linked to "proteins, polypeptides, nucleosides, nucleotides and polymers." This is not a unique property of the present compounds or the compounds disclosed in the '852 patent. The unique structural,

spectral properties and the presently claimed compounds ability to form protein conjugates are considered novel and non-obvious in view of the '852 patent by the Applicants.

Finding of prima facie obviousness—rational and motivation (MPEP §2142-2413)

One of ordinary skill in the art would have been motivated to make the claimed compounds with the expectation that additional compounds useful for the dyeing of nucleotides and in polynucleotide sequencing would be obtained.

Applicants respectfully assert that the Examiner has not demonstrated that the presently claimed compounds are *prima facie* obvious in view of the '852 patent. The currently amended present claims contain claim limitations that are not disclosed or taught by the '852 patent. The limitations include the 5-membered heteroaromatic lower fused ring structures wherein the ring comprises an O, S, or N atom.

Applicants respectfully assert that a *prima facie* case of obviousness has not been made because 1) the '852 patent does not teach or motivate one of skill in the art to modify the disclosed compounds to arrive at the presently claimed compounds, 2) because the '852 patent does not teach the presently claimed compounds there is no reasonable expectation of success and 3) the '852 patent does not contain all of the present claim limitations. Furthermore, the Applicants discovered that the presently claimed compounds comprising a heteroatom in the lower fused rings shifted the spectra emission to at least 20nm beyond known Rhodamine compounds. The '852 patent (*See*, Example 4) accomplished this by adding chloro groups to the compounds, this is a know way to shift the emission spectra of fluorescent compounds. This shift of the presently claimed compound is advantageous because these compounds can be excited in the 400-600nm range and are compatible with commercially available HeNe laser for an emission spectra of 633nm (page 1 lines 26-31: page 2 lines 1-3).

Applicants respectfully request that the Examiner withdraw the obviousness rejection of Claims 22, 24, 25 and 28 to 33 over the '852 patent.

RESPONSE TO THE OBJECTIONS

In the response that follows, the Examiner's individual objections are provided in full text, as identified by indented small bold print, followed by the Applicant's response.

Claims 26, 27 and 34 to 39 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Applicants believe that they have over come the rejections to the base claims and are therefore choosing at this time not to rewrite the listed dependent claims as independent claims.

CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (541) 984-5656.

Respectfully submitted,

Date: May 22, 2003

Koren J. Anderson

Koren J. Anderson, Ph.D.
Reg. No. 51,061

Molecular Probes, Inc.
P.O. Box 22010
Eugene, Oregon, 97402
Phone: (541) 984-5656
Facsimile: (541) 465- 8354

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Diwu *et al.*	
Serial No.: 09/922,333	Examiner: F. Powers
Filed: August 4, 2001	Group Art Unit: 1626
For: **DERIVATIVES OF 1,2-DIHYDRO-7-HYDROXYQUINOLINES CONTAINING FUSED RINGS**	**MARKED-UP VERSION OF THE CLAIMS**

Assistant Commissioner for Patents
U.S. Patent and Trademark Office
Washington, D.C. 20231

Dear Sir:

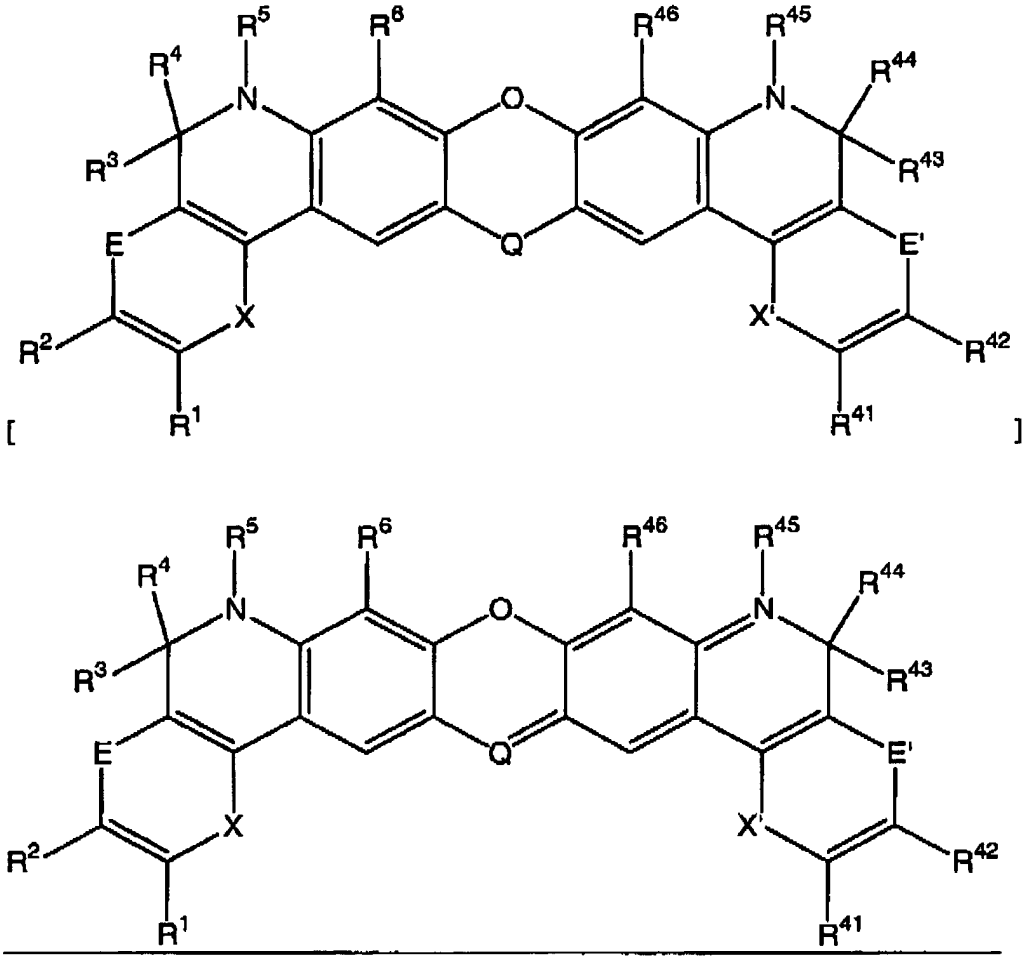
The following Marked-up Version of the Claims is hereby submitted together with the Response to Office Action on or before the due date of May 26, 2003.

CERTIFICATE OF TRANSMISSION

I HEREBY CERTIFY THAT THIS PAPER AND THE DOCUMENTS REFERRED AS BEING ATTACHED OR ENCLOSED HEREWITH ARE BEING FACSIMILE TRANSMITTED TO THE UNITED STATES PATENT AND TRADMARK OFFICE ON 5/22/03 TO 1.703.872.9306 By Michael A. Sennett

1-21 (Withdrawn)

22. (Currently Amended) A compound [comprising a] of the formula:



wherein R^1, R^2, R^6 R^{41}, R^{42}, and R^{46} are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid C_1-C_6 alkyl, C_1-C_6 alkoxy, aryl, heteroaryl, -L-R_x and -L-S_C, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is

optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or R^1 in combination with R^2, or R^{41} in combination with R^{42}, or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by -L-R_x or -L-S_C;

R^3, R^4, R^{43}, and R^{44} are independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, an aromatic or heteroaromatic ring, L-R_x and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R^2 in combination with R^3, or R^{42} in combination with R^{43}, or R^3 in combination with R^4, or R^{43} in combination with R^{44}, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

R^5 and R^{45} are independently selected from the group consisting of hydrogen, methyl, carboxymethyl, C_2-C_6 alkyl, aryl, heteroaryl, -L-R_x and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

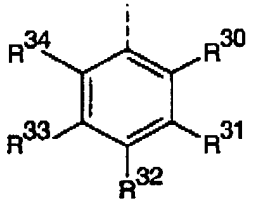
or R^4 in combination with R^5, or R^5 in combination with R^6, or R^{44} in combination with R^{45}, or R^{45} in combination with R^{46}, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

wherein one of said E, E', X' and X is O, S, or NR^8[, or $CR^{1'}$=$CR^{2'}$] provided that E and X or E' and X' are not both present;

wherein R^8 is independently selected from the group consisting of hydrogen, methyl, carboxymethyl, C_2-C_6 alkyl, -L-R_X and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; [and]

[$R^{1'}$ and $R^{2'}$ are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid, C_1-C_6 alkyl, C_1-C_6 alkoxy, aryl, heteroaryl, -L-R_X and -L-S_C, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;]

Q is N or CR^{28}, wherein R^{28} is independently selected from the group consisting of hydrogen, F, CN, carboxylic acid, a carboxylic acid ester of a C_1-C_6 alcohol, a C_1-C_6 alkyl, -L-R_x and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R^{28} comprises a formula



wherein R^{30}, R^{31}, R^{32}, R^{33} and R^{34} are independently selected from the group consisting of hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino, C_1-C_{18} alkyl, C_1-C_{18} alkoxy, C_1-C_{18} alkylthio, C_1-C_{18} alkanoylamino, C_1-C_{18} alkylaminocarbonyl, C_2-C_{36} dialkylaminocarbonyl, C_1-C_{18} alkyloxycarbonyl, C_7-C_{18} arylcarboxamido, -L-R_x and -L-S_C, wherein said alkyl or aryl portions of said R^{30}, R^{31}, R^{32}, R^{33} and R^{34} are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C_1-C_6 alcohol, sulfonic acid, amino, C_1-C_6 alkylamino, C_2-C_6 dialkylamino and C_1-C_6 alkoxy; or a pair of adjacent R^{30}, R^{31}, R^{32}, R^{33} and R^{34} substituents when taken in

combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; and

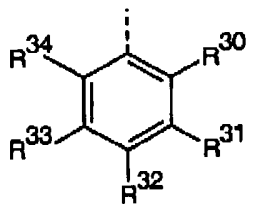
wherein L is a covalent linkage;

R_x is a reactive group; and

S_C is a conjugated substance.

23. (Cancelled)

24. (Previously Amended) The compound according to Claim 22, wherein Q is CR^{28} and R^{28} has the formula



25. (Previously Amended) The compound according to Claim 24, wherein one of R^5, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, and R^{45} is -L-R_x or -L-S_C.

26. (Previously Amended) The compound according to Claim 24, wherein

said R^3, R^4, R^{43}, and R^{44} are each methyl;

each R^1 and R^{41} is independently H or sulfonic acid; and

R^6 and R^{46} are H.

27. (Previously Amended) The compound according to Claim 24, wherein said

compound is substituted one or more times by sulfonic acid.

28. (Previously Amended) The compound according to Claim 22, wherein one of said R^1, $R^{1'}$, R^2, $R^{2'}$, R^3, R^4, R^5, R^6, R^{28}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{41}, R^{42}, R^{43}, R^{44}, R^{45}, and R^{46} is -L-R_x or -L-S_C.

29. (Previously Amended) The compound according to Claim 28, wherein each L is independently a single covalent bond, or L is a covalent linkage having 1-20 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S.

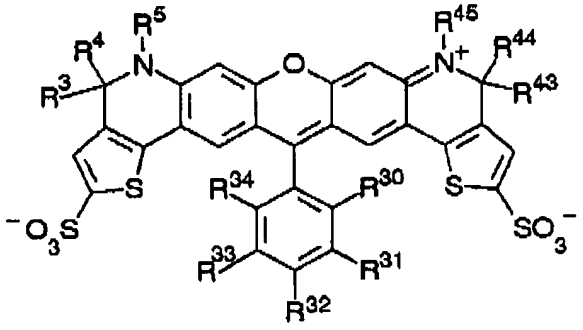
30. (Previously Amended) The compound according to Claim 28, wherein said R_X is independently selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an amine, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a carboxylic acid, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a sulfonyl halide, a perfluorobenzamido, an azidoperfluorobenzamido group, and a thiol group.

31. (Previously Amended) The compound according to Claim 30, wherein said R_X is independently selected from the group consisting of a phosphoramidite, a succinimidyl ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, a perfluorobenzamido, an azidoperfluorobenzamido group, and a reactive platinum complex.

32. (Previously Amended) The compound according to Claim 28, wherein said S_C is independently selected from the group consisting of an amino acid, a peptide, a protein, a tyramine, a carbohydrate, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a polymer, a polymeric microparticle, a biological cell, and a virus.

33. (Previously Amended) The compound according to Claim 32, wherein S_C is an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, and a nucleic acid.

34. (Currently Amended) The compound according to Claim 28, wherein said compound comprises a formula:



wherein said R^3, R^4, R^5, R^{43}, R^{44} , and R^{45} are independently methyl or ethyl;
R^{30} is sulfonic acid or carboxylic acid;
R^{31} and R^{34} are independently H, F, or Cl;
one of R^{32} and R^{33} is H, F, or Cl, and the other of R^{32} and R^{33} is -L-R_x or -L-S_c,

wherein said L is a covalent linkage comprising $-S(CH_2)_aCOO(CH_2)_b-$ or $-S(CH_2)_aCONH(CH_2)_b-$

wherein a is an integer between 0 and 10, and b is an integer between 0 and 10; and

wherein said R_x is selected from the group consisting of a carboxylic acid, an activated ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, and a reactive platinum complex[.]; and

wherein said S_c is selected from the group consisting of an amino acid, a peptide, a protein, an ion-complexing moiety, a nucleoside, a nucleotide, an oligonucleotide, a lectin, or a nucleic acid.

35. (Currently Amended) The compound according to Claim 34, wherein said R_x is a maleimide group or is a [succcinimidyl] succinimidyl ester of a carboxylic acid.

36. (Previously Amended) The compound according to Claim 34, wherein said S_c is a peptide or a protein.

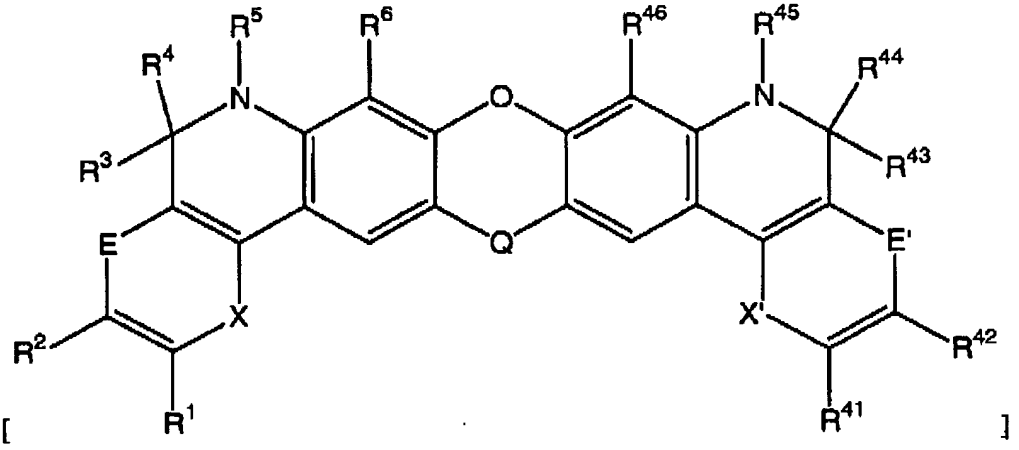
37. (Previously Amended) The compound according to Claim 36, wherein said S_c is an antibody or antibody fragment or a lectin.

38. (Previously Amended) The compound according to Claim 34, wherein said S_c is a nucleotide or an oligonucleotide.

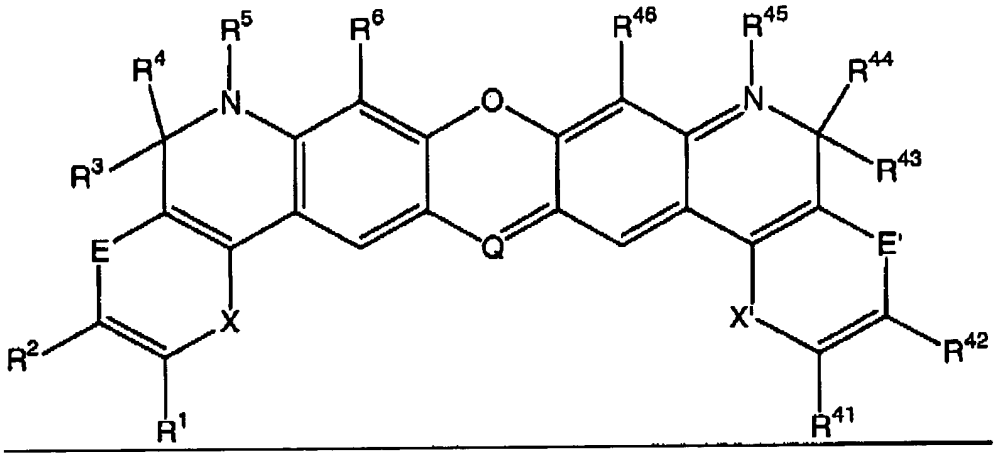
39. (Previously Amended) The compound according to Claim 34, wherein said S_c is a BAPTA or APTRA ion-complexing moiety.

40. (Currently Amended) A method of staining a sample, said method comprising steps:

a) combining a solution with said sample, wherein said solution comprises a compound having formula



[ ]



wherein R^1, R^2, R^6, R^{41}, R^{42}, and R^{46} are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid C_1-C_6 alkyl, C_1-C_6 alkoxy, aryl, heteroaryl, -L-R_x and -L-S_C, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or R^1 in combination with R^2, or R^{41} in combination with R^{42}, or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by -L-R_X or -L-S_C;

R^3, R^4, R^{43}, and R^{44} are independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, an aromatic or heteroaromatic ring, L-R_x and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R^2 in combination with R^3, or R^{42} in combination with R^{43}, or R^3 in combination with R^4, or R^{43} in combination with R^{44}, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

R^5 and R^{45} are independently selected from the group consisting of hydrogen, methyl, carboxymethyl, C_2-C_6 alkyl, aryl, heteroaryl, -L-R_x and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

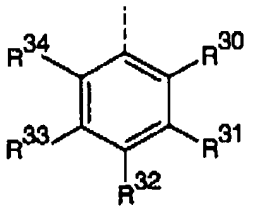
or R^4 in combination with R^5, or R^5 in combination with R^6, or R^{44} in combination with R^{45}, or R^{45} in combination with R^{46}, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

wherein one of said E, E', X' and X is O, S, or NR^8[, or $CR^{1'}$=$CR^{2'}$] provided that E and X or E' and X' are not both present;

wherein R^8 is independently selected from the group consisting of hydrogen, methyl, carboxymethyl, C_2-C_6 alkyl, -L-R_X and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; [and]

[$R^{1'}$ and $R^{2'}$ are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid, C_1-C_6 alkyl, C_1-C_6 alkoxy, aryl, heteroaryl, -L-R_X and -L-S_C, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;]

Q is N or CR^{28}, wherein R^{28} is independently selected from the group consisting of hydrogen, F, CN, carboxylic acid, a carboxylic acid ester of a C_1-C_6 alcohol, a C_1-C_6 alkyl, -L-R_x and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R^{28} comprises a formula



wherein R^{30}, R^{31}, R^{32}, R^{33} and R^{34} are independently selected from the group consisting of hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazino, C_1-C_{18} alkyl, C_1-C_{18} alkoxy, C_1-C_{18} alkylthio, C_1-C_{18} alkanoylamino, C_1-C_{18} alkylaminocarbonyl, C_2-C_{36} dialkylaminocarbonyl, C_1-C_{18} alkyloxycarbonyl, C_7-C_{18} arylcarboxamido, -L-R_x and -L-S_C, wherein said alkyl or aryl portions of said R^{30}, R^{31}, R^{32}, R^{33} and R^{34} are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C_1-C_6 alcohol, sulfonic acid, amino, C_1-C_6 alkylamino, C_2-C_6 dialkylamino and C_1-C_6 alkoxy; or a pair of adjacent R^{30}, R^{31}, R^{32}, R^{33} and R^{34} substituents when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; and

wherein L is a covalent linkage;

R_x is a reactive group; and

S_C is a conjugated substance;

b) illuminating said sample with a suitable light wavelength to yield a detectable optical response.

41. (Previously Amended) The method according to Claim 40, wherein said method further comprises combining said sample with an additional detection reagent.

42. (Cancelled)

43. (Previously Amended) The method according to Claim 40, wherein said sample comprises cells, growth medium, tissue, proteins, peptides, or biological fluids.

44. (Previously Amended) The method according to Claim 40, wherein said sample is immobilized in or on a solid or semi-solid matrix that is a membrane, an electrophoretic gel, a silicon chip, a glass slide, a microwell plate, or a microfluidic chip.

45. (Cancelled)

46. (Previously Amended) The method according to Claim 40, wherein at least one of said R^{28}, R^{30}, R^{31}, R^{32}, R^{33}, and R^{34} is -L-R_x or -L-S_C;

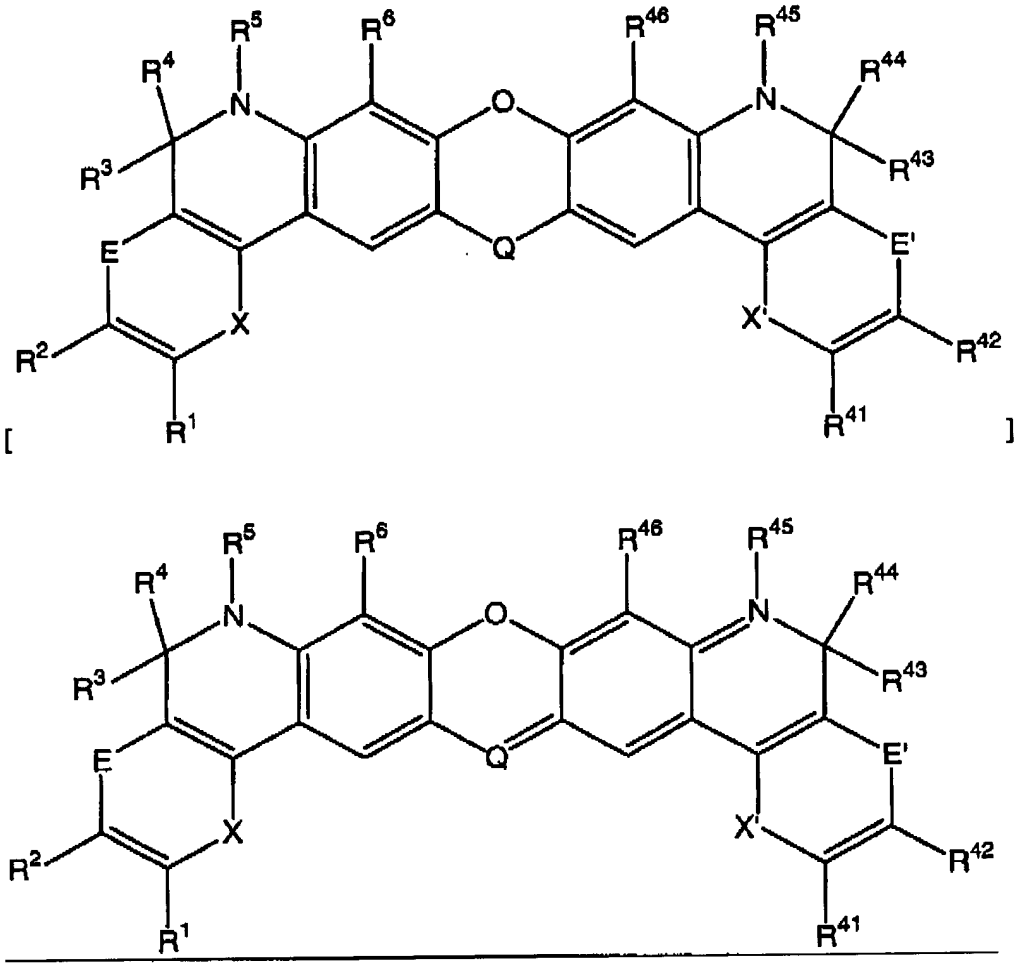
R_x is selected from the group consisting of a carboxylic acid, an activated ester of a carboxylic acid, an amine, an azide, a hydrazine, a haloacetamide, an alkyl halide, an isothiocyanate, and a maleimide group; and

S_c is selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleotide, a nucleoside, an oligonucleotide, a nucleic acid polymer, an ion-complexing moiety, a lipid, or a non-biological organic polymer or polymeric microparticle, wherein said Sc is optionally bound to one or more additional fluorophores.

47. (Previously Amended) The method according to Claim 46, wherein said R^{28} is an -L--S_C, and S_C is an ion-complexing moiety that is a BAPTA or an APTRA.

48. (Previously Amended) The method according to Claim 46, wherein at least one of said R^{28}, R^{30}, R^{31}, R^{32}, R^{33}, and R^{34} is -L-S_C, and said S_C is a nucleoside, a nucleotide, an oligonucleotide, or a nucleic acid polymer.

49. (Currently Amended) A kit for staining a sample, wherein said kit comprises
a solution comprising a buffer and a compound having formula



wherein R^1, R^2, R^6, R^{41}, R^{42} and R^{46} are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid C_1-C_6 alkyl, C_1-C_6 alkoxy, aryl, heteroaryl, -L-R_x and -L-S_C, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;

or R^1 in combination with R^2, or R^{41} in combination with R^{42}, or both, forms a fused aromatic or heteroaromatic ring that is optionally sulfonated one or more times, or said ring is substituted by -L-R_X or -L-S_C;

R^3, R^4, R^{43}, and R^{44} are independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, an aromatic ring, a heteroaromatic ring, L-R_x and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, hydroxy, or halogen and said aromatic or heteroaromatic ring is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 alkoxy, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

or R^2 in combination with R^3, or R^{42} in combination with R^{43}, or R^3 in combination with R^4, or R^{43} in combination with R^{44}, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

R^5 and R^{45} are independently selected from the group consisting of hydrogen, methyl, carboxymethyl, C_2-C_6 alkyl, aryl, heteroaryl, -L-R_x and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, carboxylic acid, sulfonic acid, or halomethyl;

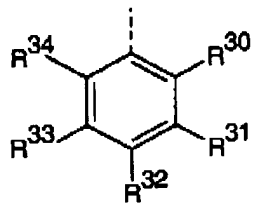
or R^4 in combination with R^5, or R^5 in combination with R^6, or R^{44} in combination with R^{45}, or R^{45} in combination with R^{46}, or any combination thereof, forms a 5- or 6-membered alicyclic ring;

wherein one of said E, E', X' and X is O, S, or NR^8[, or $CR^{1'}=CR^{2'}$], provided that E and X or E' and X' are not both present;

wherein R^8 is independently selected from the group consisting of hydrogen, methyl, carboxymethyl, C_2-C_6 alkyl, -L-R_X and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; and

[$R^{1'}$ and $R^{2'}$ are independently selected from the group consisting of hydrogen, cyano, halogen, carboxylic acid, sulfonic acid, C_1-C_6 alkyl, C_1-C_6 alkoxy, aryl, heteroaryl, -L-R_X and -L-S_C, wherein said alkyl or alkoxy is optionally substituted by carboxylic acid, sulfonic acid, or halogen and said aryl or heteroaryl is optionally substituted one or more times by C_1-C_6 alkyl, C_1-C_6 perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl;]

Q is N or CR^{28}, wherein R^{28} is independently selected from the group consisting of hydrogen, F, CN, carboxylic acid, a carboxylic acid ester of a C_1-C_6 alcohol, a C_1-C_6 alkyl, -L-R_X and -L-S_C, wherein said alkyl is optionally substituted by carboxylic acid, sulfonic acid, amino, or halogen; or R^{28} comprises a formula



wherein R^{30}, R^{31}, R^{32}, R^{33} and R^{34} are independently selected from the group consisting of hydrogen, F, Cl, Br, I, sulfonic acid, carboxylic acid, CN, nitro, hydroxy, azido, amino, hydrazine, C_1-C_{18} alkyl, C_1-C_{18} alkoxy, C_1-C_{18} alkylthio, C_1-C_{18} alkanoylamino, C_1-C_{18} alkylaminocarbonyl, C_2-C_{36} dialkylaminocarbonyl, C_1-C_{18} alkyloxycarbonyl, C_7-C_{18} arylcarboxamido, -L-R_x and -L-S_C, wherein said alkyl or aryl portions of said R^{30}, R^{31}, R^{32}, R^{33} and R^{34} are optionally substituted one or more times by substituents selected from the group consisting of F, Cl, Br, I, hydroxy, carboxylic acid, a carboxylic acid ester of a C_1-C_6 alcohol, sulfonic acid, amino, C_1-C_6 alkylamino, C_2-C_6 dialkylamino and C_1-C_6 alkoxy; or a pair of adjacent R^{30}, R^{31}, R^{32}, R^{33} and R^{34} substituents when taken in combination, form a fused 6-membered aromatic ring that is optionally further substituted by carboxylic acid; and

wherein L is a covalent linkage;

R_x is a reactive group; and

S_c is a conjugated substance.

50. (Previously Added) The kit according to Claim 49, wherein said kit further comprises an additional detection reagent, a purification medium, or standards.

51. (Previously Added) The kit according to Claim 49, wherein at least one of said R^1, R^2, R^3, R^4, R^5, R^6, R^{28}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{43}, R^{44}, R^{45} and R^{46} is L-R_x wherein said R_x is independently selected from the group consisting of a carboxylic acid, an activated ester of a carboxylic acid, an amine, an azide, a hydrazine, a haloacetamide, an alkyl halide, an isothiocyanate, and a maleimide group.

52. (Previously Added) The kit according to Claim 51, wherein at least one of said R^{31}, R^{32}, R^{33}, or R^{34} is L-R_x and R^{30} is carboxylic acid or sulfonic acid.

53. (Previously Added) The kit according to Claim 49, wherein at least one of said R^1, R^2, R^3, R^4, R^5, R^6, R^{28}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{43}, R^{44}, R^{45} and R^{46} is L-Sc, wherein said Sc is independently selected from the group consisting of an amino acid, a peptide, a protein, an antibody, an antibody fragment, a carbohydrate, a nucleotide, a nucleoside, an oligonucleotide, a nucleic acid polymer, an ion-complexing moiety, a lipid, a non-biological organic polymer and polymeric microparticle.

54. (Previously Added) The kit according to Claim 53, wherein said Sc is an antibody or fragment thereof.

Diwu *et al.*
Serial No. 09/922,333

Docket No. D305.001PN.2

Respectfully submitted,

Date: May 22, 2003

Koren J. Anderson, Ph.D.
Reg. No. 51,061

Molecular Probes, Inc.
P.O. Box 22010
Eugene, Oregon, 97402
Phone: (541) 984-5656
Facsimile: (541) 465- 8354

AUTOMATIC COVER SHEET

DATE: MAY-22-2003 THU 03:14 PM

TO:

FAX #: 917038729306

FROM: MOLECULAR PROBES INC

FAX #: 15414658354

29 PAGES WERE SENT

(INCLUDING THIS COVER PAGE)